Janus Kinases: An Ideal Target for the Treatment of Autoimmune Diseases

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Cytokines have pivotal roles in the maintenance of an appropriate immune response. Targeting cytokine receptors has been an effective means of treating immune-related disorders. In the past few years, research efforts have been directed toward cytokines' intracellular signaling pathways and, in particular, the JAK-STAT (Janus kinase-signal transducers and activation of transcription) signaling cascade. Recently, spearheaded by the development of effective drugs in cancer treatment, it has become clear that the targeting of intracellular protein kinases is a very attractive and feasible possibility for the treatment of autoimmune disorders. The targeting of the Janus kinases (JAKs) has been quite successful and two JAK inhibitors are now approved to be used in humans. Interestingly, although some of the inhibitors developed and tested to date have been shown to target more than one kinase, this promiscuity does not appear to be problematic. Novel second-generation, more specific inhibitors are under development, and in the next few years, we expect this class of drugs to become a powerful tool in the hands of clinician treating autoimmune diseases.

Journal of Investigative Dermatology Symposium Proceedings (2013) **16,** S70–S72; doi:10.1038/jidsymp.2013.29

Cytokines are soluble factors with critical functions in several biological responses. In particular, they serve as an intracellular communication tool of immune system, and their release and actions help shape the immune response. As a result, when these molecules are produced in abnormal amounts, be these higher or lower, the homeostasis of the immune system is altered and several pathologies ensue (O'Shea *et al.*, 2011). Autoimmune disorders are a classical example of such pathologies as several proinflammatory cytokines have been demonstrated to drive such diseases (Xavier and Rioux, 2008). It was not surprising then that targeting cytokines and their receptors resulted in the development of several drugs currently utilized to treat autoimmune diseases. The class of drugs known as biologics, which includes mAbs, recombinant soluble receptors, and fusion proteins of receptor moieties with antibodies' constant fragments, has, in the past 15 years, completely revolutionized the clinical approach to the treatment of immune disorders (Strand *et al.*, 2007).

Like any other drugs, biologics are not magic bullets and their use has some limitations. First of all, these drugs have to be administered parenterally. Moreover, being proteins in nature, they often have high molecular weight and therefore do not cross the blood–brain barrier and cannot function within the central nervous system. Importantly, some patients are refractory to their effects and the high cost is an issue in some countries.

Like many soluble factors, cytokines bind to their receptors on the surface of cells and trigger signaling events that involve several cytosolic substrates. These substrates would be ideal targets for the development of small molecules aiming at modulating cellular responses. On the other hand, because of the complexity and the intricacies of signaling pathways, the goal is not easily achievable.

In the case of cytokines, the activation of the tyrosine kinase of the Janus family, better known as JAKs, were shown to be a critical step. This family comprises four molecules, namely JAK1, JAK2, JAK3, and TYK2. Upon binding of cytokines to their cognate receptors, JAKs, which work in pairs, become enzymatically active and phosphorylate themselves, the receptor chains, and several other substrates including the signal transducers and activation of transcription family of latent transcription factors (Leonard and O'Shea, 1998) (Figure 1).

Cytokines act on different cells, and likewise, JAKs are expressed in many cell types. On the other hand, JAK3 is selectively expressed in hematopoietic cells and mutation in this kinase resulted in loss of function and severe-combined immunodeficiency in humans (Leonard and O'Shea, 1998). It was therefore hypothesized that blocking the enzymatic activity of JAK3 would also result in immunosuppression. Importantly, mutations in JAK2 have also been reported in humans but this resulted in a gain of function as cells expressing the mutant JAK2 proliferate in a cytokine-independent manner (Kralovics *et al.*, 2005).

Small molecules such as imatinib, which blocked the ATP-binding activity of tyrosine kinases, were successfully

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Abbreviations: JAK, Janus kinase; STAT, signal transducers and activation of transcription

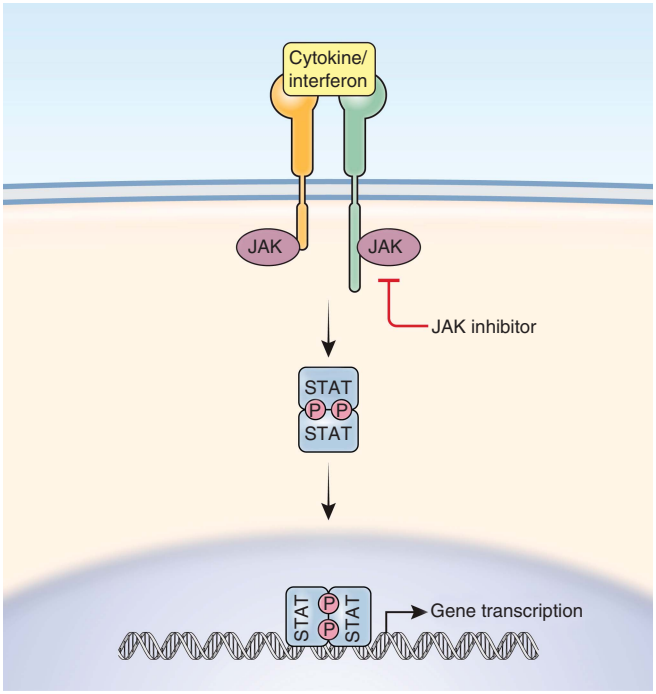

Figure 1. Janus kinase (JAK) inhibitors block JAKs enzymatic activity activation. Cytokines or interferons bind their cognate receptors and initiate a signaling cascade leading to the activation of the latent transcription factors signal transducers and activation of transcription factors (STAT) and ultimately gene transcription. JAK inhibitors interfere with this pathway by blocking JAKs' enzymatic activity.

generated and have been used in the treatment of several malignancies including leukemia, lymphomas, and even some solid tumors. Therefore, development of specific JAK inhibitors was not only desirable but also feasible. Twenty years after the discovery of the JAKs and the definition of their role in cytokine signaling, this has now become a reality.

Recently, two small molecules that inhibit JAKs' enzymatic activity have been approved for clinical use. Ruxolitinib (trade name Jakafi) is a JAK2/JAK1 inhibitor (with some activity on JAK3 and TYK2) currently prescribed for the treatment of intermediate- or high-risk myeloproliferative disorders including primary myelofibrosis post-polycythemia vera myelofibrosis and post-essential thrombocythemia myelofibrosis (Harrison *et al.*, 2012).

Tofacitinib (trade name Xeljianz) instead is a JAK3/JAK1 inhibitor (but JAK2 is also affected, albeit to a lesser extent) recently approved for the treatment of rheumatoid arthritis in patients for whom methotrexate therapy was not efficacious. In these cases, tofacitinib is used either as monotherapy or it can be combined with methotrexate or other non-biologic disease-modifying antirheumatic drugs (Fleischmann *et al.*, 2012; van Vollenhoven *et al.*, 2012).

Both these first-generation JAK inhibitors block the enzymatic activity of all the JAKs with different degrees of specificity. *In vitro* studies have shown that, with regard to the human kinome, their activity is exquisitely limited to the JAKs (Karaman *et al.*, 2008). This specificity limits their off-target effects but does not impair their capacity to blunt the effects of multiple cytokines. In fact, in the case of tofacitinib, the capacity to inhibit the actions of several proinflammatory cytokines and to act on different immune cells is possibly out of the reasons why this drug has been so efficacious in rheumatoid arthritis, a disease that pathophysiology involves the action of several cytokines.

Blocking cytokines such as interleukins, interferons, and erythropoietin results in effects on many cell types such as T, B, natural killer cells, and erythrocytes. Administration of a JAK2 inhibitor like ruxolitinib results in anemia and thrombocytopenia, as expected by the well-known role of JAK2 in erythropoietin and thrombopoietin signal transduction. On the other hand, patients receiving the drug showed clinical improvement in all the myelofibrosis-related symptoms (Mascarenhas and Hoffman, 2012).

In the case of tofacitinib, the total number of circulating T cells is not impaired but differentiation of T helper cells such as T helper type 1, T helper type 2, and T helper type 17 is impaired (Ghoreschi *et al.*, 2011). Animal studies have also shown a sharp decline in numbers of natural killer cells. Patients treated with tofactinib tended to be more prone to infections, which included opportunistic pathogens and herpes zoster. The above-mentioned effect on natural killer cells does not appear to correlate with increased incidence of tumors but long-term effects have not yet been evaluated.

Overall, JAK inhibitors' side effects partially overlap with what has been observed for biologics. Increased lipids levels, observed in patients receiving JAK inhibitors, have also been reported in patients treated with the anti-IL-6 antibody tocilizumab. Similarly, increased creatinine and transaminase levels were detected but it remains unclear if such alterations or the increase in lipids are directly related to JAK enzymatic activity.

Several clinical trials with other JAK inhibitors are currently ongoing for a wide array of diseases ranging from immune-mediated diseases, such as psoriasis, and inflammatory bowel disease to malignancies like myeloid leukemias and multiple myeloma. JAK inhibitors are also being tested as immunosuppressants in transplantation.

Some of the new, ''second-generation'' inhibitors appear to be more specific. For example, GLPG0634, currently developed by Galapagos has been reported to be JAK1-specific, whereas Vertex's VX-509 is JAK3-selective. However, it is not known whether this selectivity will result in increased efficacy. Nonetheless, we are tempted to speculate that these compounds may be better suited for diseases in which only one or very few cytokines are implicated. The mechanism of delivery (e.g. orally, parenterally, or topically) also needs to be considered with regard to their use.

In conclusion, targeting of JAKs was pursued because of their expression pattern and association with several pathologies. We can now say that inhibition of JAK enzymatic activity has clearly proved successful for some immune-related diseases, whereas other immune-related pathologies will likely soon benefit from this class of drugs.

CONFLICT OF INTEREST

MG's employer is currently collaborating with Pfizer for the development of JAK inhibitors. The author states no conflict of interest.

ACKNOWLEDGMENTS

Funding for the Summit and publication of this article was provided by the National Alopecia Areata Foundation.

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